CLAIM AMENDMENTS

IN THE CLAIMS:

1.-23. (cancelled)

24. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50 °C (at a pH of about 7.5)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Isoelectric point: about 4.5.

25. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Isoelectric point: about 4.5.

26. (canceled)

27. (previously presented) The creatine amidinohydrolase of claim 24, which has the following physicochemical properties:

Optimum pH: about 8.0-9.0 (at a temperature of about 37 °C).

28. (previously presented) The creatine amidinohydrolase of claim 24, which has a molecular weight of about 43,000 (SDS-PAGE).

29. (canceled)

30. (previously presented) The creatine amidinohydrolase of claim 25, which has a molecular weight of about 43,000 (SDS-PAGE).

31.-32. (canceled)

33. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C (at a pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point of 4.5.

34. (canceled)

35. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

36. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

37. (previously presented) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

38. (previously presented) A method for producing the creatine amidinohydrolase of claim 24, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. (previously presented) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 24, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. (previously presented) A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. (previously presented) A reagent for determination of creatinine in a sample, comprising a creatinine amidinohydrolase, the creatine amidinohydrolase of claim 24, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. (previously presented) A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.

43. (new) A method for producing the creatine amidinohydrolase of claim 25, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

44. (new) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 25, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

45. (new) A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 44 with the sample.

46. (new) A reagent for determination of creatinine in a sample, comprising a creatinine amidinohydrolase, the creatine amidinohydrolase of claim 25, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

47. (new) A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 46 with the sample.

48. (new) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50°C (at a pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5 - 10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

49. (new) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50°C (at a pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

50. (new) The creatine amidinohydrolase of claim 49, which is obtained from *Escherchia coli* JM109 (pCRH273M2) (FERM BP-5375).

51. (new) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50°C (at a pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

52. (new) The creatine amidinohydrolase of claim 51, which is obtained from *Escherchia coli* JM109 (pCRH273M1) (FERM BP-5374).

53. (new) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O $\rightarrow$ sarcosine + urea

Optimum temperature: about 40-50°C (at a pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

54. (new) The creatine amidinohydrolase of claim 53, which is obtained from *Escherchia coli* JM109 (pCRH273M3) (FERM BP-5376).

55. (new) A method for producing the creatine amidinohydrolase of claim 48, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

56. (new) The method of claim 55, wherein said microorganism is selected from the group consisting of *Escherchia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherchia coli* JM109 (pCRH273M2) (FERM BP-5375), *Escherchia coli* JM109 (pCRH273M3) (FERM BP-5376).

57. (new) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

58. (new) The reagent of claim 57, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore, and a buffer.

59. (new) The reagent of claim 58, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

60. (new) The reagent of claim 58, in which the chromophore comprises a hydrogen receptor and a coupler.

61. (new) The reagent of claim 60, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

62. (new) The reagent of claim 60, in which the coupler is an aniline derivative or a phenol derivative.

63. (new) A method for determining creatine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 49 with the sample.

64. (new) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

65. (new) The reagent of claim 64, in which the composition for the detection of hydrogen peroxide comprises and enzyme having a peroxidase activity, a chromophore, and a buffer.

66. (new) The reagent of claim 65, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

67. (new) The reagent of claim 65, in which the chromophore comprises a hydrogen receptor and a coupler.

68. (new) The reagent of claim 67, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

69. (new) The reagent of claim 67, in which the coupler is an aniline derivative or a phenol derivative.

70. (new) A method for determining creatinine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 64 with the sample.